REMARKS

Claims 22-26 are pending in this application. Claim 22 is amended herein for purposes of grammatical clarity. No new matter is added by amendment of claim 22.

Applicants respectfully request that the Examiner consider the following remarks in response to the Office Action.

Priority Determination:

The Examiner states that Applicants have not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. § 119 (e) or § 120. Specifically, the Examiner notes that "[a]n application in which the benefits of an earlier application are desired must contain a specific reference to the prior application(s) in the first sentence of the specification of (sic) in an application data sheet (37 CFR 1.78(a)(2) and (a)(5))."

Applicants have herein amended the specification to recite the priority claim reflected on the filing receipt. According to the United States Patent and Trademark Office Official Gazette Notice of 18 March 2003 (enclosed as Exhibit A for the convenience of the Examiner), such an amendment is proper:

> The reference required by 37 CFR 1.78(a)(2) or (a)(5) must be included in an application data sheet (37 CFR 1/76), or the specification must contain, or be amended to contain, such reference in the first sentence following the title. Previously the Office indicated that if an applicant includes a benefit claim in the application but not in the manner specified by 37 CFR 1.78 (a) (e.g., if the claim is included in an oath or declaration or the application transmittal letter) within the time period set forth in 37 CFR 1.78(a), the Office will not require a petition under 37 CFR 1.78(a) and the surcharge under 37 CFR 1.17(t) to correct the claim if the information concerning the claim was recognized by the Office as shown by its inclusion on the filing receipt. . . . The Office will continue to follow this practice. (emphasis added).

Applicants respectfully submit that they have satisfied each of the above requirements for recognizing an improper priority claim without requiring either the petition set forth in 37 CFR 1.78(a) or the fee under 37 CFR 1.17(t). First, Applicants included a claim for

priority within the time period specified by 37 CFR 1.78(a)(2) by including a request in the application transmittal letter submitted with the present application on October 1, 2003 to "[a]mend the specification by inserting before the first line the sentence . . . " setting forth the priority claim for this application. Second, the filing receipt for this application lists each application, and its relationship to the other applications, to which Applicants attempted to claim priority in the transmittal letter. Finally, Applicants have herein requested that the specification be amended to include the priority claim originally set forth in the application transmittal letter.

Hence, Applicants respectfully request that the Office accept Applicants' priority claim without requiring either the petition as set forth in 37 CFR 1.78(a)(2) or the fee as set forth in 37 CFR 1.17(t) and amend the specification as requested in this response and request for reconsideration.

Oath/Declaration Determination:

The Examiner has noted that the oath or declaration is defective because of non-initialed and/or non-dated alterations that have been made to the oath or declaration. Concurrent with submitting this response and request for reconsideration, Applicants have submitted a request to correct inventorship. The inventorship for this application no longer includes Dan Eaton and therefore, Applicants respectfully submit that this objection is overcome. Applicants respectfully request that the Examiner withdraw this objection.

Rejection under 35 U.S.C. § 101:

Claims 22-26 stand rejected under 35 U.S.C. §101 as allegedly not supported by either an asserted utility that is specific and substantial utility or a well-established utility. Applicants respectfully disagree with this ground of rejection. In particular, Applicants maintain that claims 22-26 are supported by the specific and substantial utility asserted at page 137 of the specification: "antagonists (*e.g.* antibodies) directed against the proteins encoded by the DNAs tested would be expected to have utility in cancer therapy and as useful

diagnostic reagents." *See also* page 119. Significantly, an Applicant's assertion of utility creates a presumption of utility that will be sufficient to satisfy the utility requirement of 35 U.S.C. § 101, "unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope." *In re Langer*, 503 F.2d 1380,1391, 183 USPQ 288, 297 (CCPA 1974). *See, also In re Jolles*, 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); *In re Irons*, 340 F.2d 974, 144 USPQ 351 (1965); *In re Sichert*, 566 F.2d 1154, 1159, 196 USPQ 209, 212-13 (CCPA 1977).

The Office action sets forth three bases upon which one of ordinary skill in the art allegedly would question Applicants' assertion of utility or its scope. First, the Office action alleges that PRO357 polypeptide, to which the claimed antibodies bind, is a "totally new, uncharacterized polypeptide with no well-established utility." Page 5 of the Office action mailed 2/27/06. Second, the Office action alleges that although the specification discloses ΔCt data indicating that the nucleic acid encoding the PRO357 polypeptide might be amplified in lung and colon tumors, that data is not persuasive because allegedly the ΔCt values were not significant enough to indicate gene amplification, the samples were not corrected for aneuploidy, and the samples where amplification was observed were not compared to normal, non-cancerous lung and colon tissue. Third, according to the Office action, "the data for PRO357 genomic DNA have no bearing on the utility of the claimed PRO357 polypeptide" because allegedly, "[i]n order for the PRO357 polypeptide to be overexpressed in lung and colon tumors, amplified genomic DNA would have to correlate with amplified mRNA, which in turn would have to correlate with amplified polypeptide levels." Page 7 of the Office action mailed 2/27/06. The Office action states that "[t]he art discloses that such correlations cannot be presumed." Page 7 of the Office action mailed 2/27/06.

Applicants respectfully disagree that one of ordinary skill in the art would reject Applicant's assertion of utility on any of the above bases. Compliance with 35 U.S.C. § 101 is a question of fact. *Raytheon v. Roper*, 724 F.2d 951, 956, 220 USPQ 592, 596 (Fed. Cir. 1983) *cert. denied*, 469 US 835 (1984). The evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the totality of the evidence under consideration. *In re Oetiker*, 977 F.2d 1443, 1445, 24

USPQ2d 1443, 1444 (Fed. Cir. 1992). Thus, to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Examiner must establish that it is more likely than not that one of ordinary skill in the art would doubt the truth of the statement of utility. Only after the Examiner made a proper *prima facie* showing of lack of utility, does the burden of rebuttal shift to the applicant. The issue will then be decided on the totality of evidence.

A prima facie case of lack of utility has not been established

None of the above bases establishes that it is more likely than not that one of ordinary skill in the art would doubt the truth of Applicants' assertion of utility. Specifically, Applicants respectfully disagree that one of ordinary skill in the art would find it more likely than not that the PRO357 polypeptide is totally uncharacterized. Rather, Applicants' have set forth a significant amount of descriptive characterization of the PRO357 polypeptide in the specification. For example, the specification discloses: the nucleic acid sequence encoding the PRO357 polypeptide, *see* Figure 25, SEQ ID NO:68; the amino acid sequence encoding the PRO357 polypeptide, *see* Figure 26, SEQ ID NO:69; several structural features of the PRO357 polypeptide, including, a signal sequence from amino acids 1-23, a transmembrane domain from amino acids 501-522, N-glycosylation sites from amino acids 198-202, 425-429, 453-457, tyrosine kinase phosphorylation sites at amino acids 262-270, N-myristoylation sites at amino acids 23-29, 27-33, 112-118, 273-279, 519-525, 565-571, a prokaryotic membrane lipoprotein lipid attachment site at amino acids 14-25, an EGF-like domain cysteine pattern signature at amino acids 355-367, and leucine zipper patterns at amino acids 122-144, and 194-216; deposit of the nucleic acid encoding the PRO357 polypeptide, *see* pages 147-148, *see also* page 107 disclosing that the nucleic acid encoding the PRO357 polypeptide, DNA44804-1248, was deposited with the ATCC as ATCC Deposit No. 209527 on December 10, 1997; how to use the PRO357 nucleic acid to express the PRO357 polypeptide in *E.coli, see* Example 20, pages 111-112, in mammalian cells, *see* Example 21, pages 112-115, in yeast, *see* Example 22, page 115, and in baculovirus-infected insect cells, *see* Example 23, pages 115-116; how to prepare antibodies that bind the PRO357 polypeptide, *see* Example 24, pages 116-117; and that

the PRO357 nucleic acid was significantly amplified in approximately 62% (26/42) of the lung and colon cancer tissue samples examined, *see* Example 28, pages 119-137, Table 10 pages 125-127. The above-identified description would lead one of ordinary skill in the art to conclude that it is more likely than not that the PRO357 polypeptide is not "uncharacterized," but rather is characterized by a variety of things, including the nucleic and amino acid sequences, deposit of the nucleic acid sequence, unique structural features of the amino acid sequence, the ability to produce the polypeptide in various systems and organism, and the fact that the genomic DNA encoding PRO357 is amplified in lung and colon tumor tissue samples.

The second basis set forth in the Office action for rejecting Applicants' assertion of utility also does not make it more likely than not that one or ordinary skill in the art would doubt Applicants' assertion of utility or question its scope. Specifically, it was well known in the art at the time the invention was made that gene amplification is an essential mechanism for oncogene activation. The gene amplification assay is well-described in Example 28 of the present application. Example 28 discloses that the inventors isolated genomic DNA from a variety of primary cancers and cancer cell lines that are listed in Table 10, including primary lung and colon tumors of the type and stage indicated in Table 9. As a negative control, DNA was isolated from the cells of ten normal healthy individuals, which was pooled and used as a control. Gene amplification was monitored using real-time quantitative TAQMAN™ PCR. Table 10 shows the resulting gene amplification data. Further, Example 28 explains that the results of TAQMAN™ PCR are reported in ΔCt units, wherein one unit corresponds to one PCR cycle or approximately a 2-fold amplification relative to control, two units correspond to 4-fold amplification, 3 units to 8-fold amplification etc. *See* page 120 of the specification. Thus, Table 10 demonstrates that gene amplification levels in 14 of the 15 (*i.e.* approximately 93%) lung tumor tissues listed in Table 9 and tested were greater than 2-fold. Specifically, amplification levels ranged from $2^{1.18} - 2^{3.51}$. Similarly, gene amplification levels in 12 of the 17 (*i.e.* approximately 71%) colon tumor tissues listed in Table 9 and tested were greater than 2-fold.

A change of at least 2-fold amplification relative to normal, or 1 ΔCt unit, is art recognized as indicative of significant levels of gene amplification. In support of this, Applicants respectfully direct the Examiner's attention to the Declaration of Audrey Goddard, Ph.D., submitted herewith as Exhibit B. Dr. Goddard, an expert in the field of cancer biology and an inventor of the present invention, clearly states in her declaration that one of ordinary skill in the art would find it more likely than not that the data set forth in Table 10 at pages 125-127 of the specification indicates that the levels of PRO357 genomic DNA would be diagnostic of lung or colon cancer. Specifically, the Goddard Declaration illustrates the art acceptance of gene amplification data as an indicator of cancerous tissue. For example, in paragraph 7, Dr. Goddard specifically asserts her opinion that:

> [a]n at least 2-fold increase in gene copy number in a tumor tissue sample relative to a normal (*i.e.* non-tumor) sample is significant and useful in that the detected increase in gene copy number in the tumor sample relative to the normal sample serves as a basis for using relative gene copy number . . . as a diagnostic marker for the presence or absence of tumor in a tissue sample of unknown pathology.

The Office action further rejects the ΔCt data set forth in Table 10 because allegedly, "[c]ancerous tissue is known to be aneuploid . . . the data presented in the specification were not corrected for aneuploidy . . . [and therefore,] amplification of a gene does not necessarily correlate with overexpression in a cancer tissue, but can merely be an indication that the cancer tissue is aneuploid." Page 6 of the Office action mailed 2/27/06.

Applicants respectfully disagree that the gene amplification data may have resulted from testing cancer tissue that was aneuploid. In particular, the gene amplification data presented in Example 28 of the specification resulted from experiments using appropriate controls for aneuploidy. For example, as explained at page 134 of the specification:

> PRO357 was reexamined with selected tumors from the above initial screen with framework mapping. Table 19 indicate[s] the chromosomal mapping of the framework markers that were used in the present example. *The framework markers are located approximately every 20 megabases and were used to control aneuploidy.* (emphasis added).

Thus, Applicants used framework mapping to control for aneuploidy and to ensure that the observed ΔCt value represented relevant gene amplification. In addition, Applicants also examined the PRO357 gene amplification using epicenter mapping, *see e.g.* pages 134-136, as well as compared the observed amplification levels to amplification levels in cultured cell lines, primary tumors, and normal human blood. Applicants therefore respectfully submit that one of ordinary skill in the art would find it more likely than not that the ΔCt data set forth in Table 10 is indicative of amplification of the PRO357 nucleic acid in lung and colon tumor tissue.

However, the Office action alleges that even if the gene amplification data were accepted as indicative of gene amplification rather than simply aneuploidy, that data still would not be supportive of utility. In particular, the third basis the Office action sets forth for rejecting Applicants' assertion of utility is that correlation between gene amplification and protein overexpression is not presumed to occur, absent actual evidence demonstrating such a correlation exists. Applicants respectfully disagree that actual evidence of a correlation between the disclosed amplification of the PRO357 nucleic acid and the asserted overexpression of the PRO357 polypeptide is required to demonstrate utility of the claimed invention. Under the proper utility standard, Applicants have demonstrated that the present invention is supported by a specific, substantial, and credible utility. Specifically, Applicants herein provide several declarations and cite several art references which indicate that one of ordinary skill in the art would not have reasonably questioned the utility asserted at pages 119 and 137 of the specification.

Significantly, statistical certainty regarding an Applicants' assertion of utility is not required to satisfy 35 U.S.C. § 101. *Nelson v. Bowler,* 626 F.2d 853, 856-857, 205 USPQ 881, 883-884 (CCPA 1980). Where an Applicant has specifically asserted that an invention has a particular utility, that assertion cannot simply be dismissed as "wrong" even where there may be some reason to question the assertion. MPEP § 2107.02. Rather, a 35 U.S.C. § 101 rejection should only be sustained where the asserted utility violates a scientific principle or is *wholly* inconsistent with contemporary

knowledge in the art. *In re Gazave,* 379 F.2d 973, 978, 154 U.S.P.Q. 92, 96 (CCPA 1967) (emphasis added).

The rejection in the present case is inappropriate because the assertion of utility based on correlation between gene amplification and protein overexpression levels does not violate any scientific principle, nor is it wholly inconsistent with knowledge in the art. Indeed, according to Genes V, a central dogma of molecular biology is that genes are perpetuated as nucleic acid sequences, but function by being expressed in the form of proteins. Thus, genetic information is perpetuated by replication where a double-stranded nucleic acid is duplicated to give identical copies. These copies are then expressed by a two-stage process. First, transcription generates a single-stranded RNA identical in sequence with one of the strands of the duplex DNA. This RNA strand is then translated such that the nucleotide sequence of the RNA is converted into the sequence of amino acids comprising a protein. *See* Lewin, Bejamin. *Genes V.* 1994. Oxford University Press, NY, NY. p. 163.

Those of skill in the art generally accept that gene expression levels correlate to protein expression levels absent specific events such as translation regulation, post-translation processing, protein degradation, protein isolating errors, etc. *See* Orntoft *et al.,* "Genome-wide study of gene copy numbers, transcripts, and protein levels in pairs of non-invasive and invasive human transitional cell carcinomas." 2002. *Molecular & Cellular Proteomics 1.1,* 37-45. Indeed, the Declaration of Paul Polakis, Ph.D., submitted herewith as Exhibit C, illustrates the art acceptance of a correlation between mRNA levels and polypeptide levels. More specifically, Dr. Polakis, principal investigator of the Tumor Antigen Project of Genentech, Inc., the assignee of the present application, further explains:

> 4. In the course of the research conducted by Genentech's Tumor Antigen Project . . . using mircroarray analysis, we have identified approximately 200 gene transcripts that are present in human tumor cells at significantly higher levels than in corresponding normal human cells. To date, we have generated antibodies that bind to about 30 of the tumor antigen proteins expressed from

these differentially expressed gene transcripts and have used these antibodies to quantitatively determine the level of production of these tumor antigen proteins in both human cancer cells and corresponding normal cells. We have then compared the levels of mRNA and protein in both the tumor and normal cells analyzed.

5. From the mRNA and protein expression analyses described in paragraph 4 above, we have observed that there is a strong correlation between changes in the level of mRNA present in any particular cell type and the level of protein expressed from that mRNA in that cell type. In approximately 80% of our observations we have found that increases in the level of a particular mRNA correlates with changes in the level of protein expressed from that mRNA when human tumor cells are compared with their corresponding normal cells.

6. Based upon my own experience accumulated in more than 20 years of research, including the data discussed in paragraphs 4 and 5 above and my knowledge of the relevant scientific literature, it is my considered scientific opinion that for human genes, an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell. In fact, it remains a central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded protein. While there have been published reports of genes for which such a correlation does not exist, it is my opinion that such reports are exceptions to the commonly understood general rule that increased mRNA levels are predictive of corresponding increased levels of the encoded protein.

Significantly, Dr. Polakis declares that "in approximately 80%" of the cases observed in connection with the Tumor Antigen Project, increases in the mRNA levels correlated with changes in the levels of protein expression. Thus, this is direct evidence of the empirical experimentation the Office action asserts would be necessary for one in the art to accept that gene amplification correlates with protein overexpression. As stated above, according to MPEP § 2107, the Examiner "must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being

questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." (emphasis added).

Moreover, Applicants herein submit as Exhibit D a second declaration by Dr. Polakis, which supplements the first declaration by providing data indicating that "of the 31 genes identified as being detectably overexpressed in human tumor tissue as compared to normal tissue at the mRNA level, 28 of them (i.e., greater than 90%) are also detectably overexpressed in human tumor tissue as compared to normal human tissue at the protein level. As such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA." *See* Paragraph 5 and Exhibit B of the Second Declaration of Paul Polakis, Ph.D. (emphasis original).

Further, numerous art references including those by Pollack, Orntoft, Hyman, Bermont, Varis, Hu, Papotti, Walmer, Janssens, Hahnel, Kammori, Bea, Maruyama, and Futcher demonstrate that the utility of the claimed antibodies is not wholly inconsistent with the knowledge in the art. In addition, these references further support Applicants' argument that one of ordinary skill in the art would reasonably find it more likely than not that the present invention is supported by a specific, substantial, and credible utility.

For example, Pollack *et al.* profiled DNA copy number alterations across 6,691 mapped human genes in 44 breast tumors and 10 breast cancer cell lines and reported that microarray measurements of mRNA levels revealed remarkable degrees to which variation in gene copy number contributes to variation in gene expression in tumor cells. *See* Pollack *et al.,* "Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors." 2002. *PNAS,* 99(20):12963-12968 (submitted herewith). Pollack et al further report that their findings that DNA copy number plays a role in gene expression levels are generalizable. Thus significantly, "[t]hese findings provide evidence that widespread DNA copy number

alteration can lead directly to global deregulation of gene expression, which may contribute to the development or progression of cancer."

In particular, Pollack et al. report a parallel analysis of DNA copy number and mRNA levels. Pollack et al. found that "[t]he overall patterns of gene amplification and elevated gene expression are *quite concordant, i.e.*, a significant fraction of highly amplified genes appear to be correspondingly highly expressed." (emphasis added). Specifically, of 117 high-level DNA amplifications 62% were associated with at least moderately elevated mRNA levels and 42% were found associated with comparably highly elevated mRNA levels.

Orntoft *et al.* report similar findings in "Genome-wide study of gene copy numbers, transcripts, and protein levels in pairs of non-invasive and invasive human transitional cell carcinomas." 2002. *Molecular & Cellular Proteomics 1.1,* 37-45 (submitted herewith). Initially, Orntoft *et al.* note that "[h]igh throughput array studies of the breast cancer cell line BT474 ha(ve) suggested that there is a correlation between DNA copy numbers and gene expression in highly amplified areas ( ), and studies of individual genes in solid tumors have revealed a good correlation between gene dose and mRNA or protein levels in the case of c-erb-B2, *cyclin d1, ems1,* and N-myc."

Specifically, Orntoft *et al.* used 2D-PAGE analysis on four breast tumor tissue samples to determine correlation between genomic and protein expression levels of 40 well resolved, known proteins. Orntoft reported that "[i[n general there was a *highly significant correlation* ($p<0.005$) between mRNA and protein alterations ( ). Only one gene showed disagreement between transcript alteration and protein alteration." (emphasis added). Additionally, Orntoft *et al.* report that "11 chromosomal regions where CGH showed aberrations that corresponded to the changes in transcript levels also showed corresponding changes in the protein level ( )." The regions examined by Orntoft include genes encoding proteins that are often found altered in bladder cancer.

Orntoft *et al.* note that their study reports a *striking correspondence* between DNA copy number, mRNA expression and protein expression. Orntoft *et al.*, further note that any observed discrepancies in correlation may be attributed to translation regulation, post-

translation processing, protein degradation or some combination of these. *See also* Hyman *et al.,* "Impact of DNA amplification on gene expression patterns in breast cancer." 2002. *Cancer Research*, 62:62-40-6245 (submitted herewith).

Varis, Bermont, Papotti, Walmer, Janssens, Hahnel, Kammori, Maruyama, and Bea are yet further examples that utility of the present invention based on a correlation between gene amplification and protein overexpression is not wholly inconsistent with knowledge in the art. Varis *et al.,* carried out a comprehensive analysis of gene copy number and expression levels of 636 chromosome 17-specific genes in gastric cancer. *See* Varis *et al.*, "Targets of gene amplification and overexpression at 17q in gastric cancer." *Cancer Res.* 2002. 1;62(9):2625-9 (submitted herewith). Specifically, Varis *et al.* report that analysis of DNA copy number changes by comparative genomic hybridization on a cDNA microarray revealed increased copy numbers of 11 genes, 8 of which were found to be overexpressed in the expression analysis. Thus, Varis *et al.*, teach there is a 72% correlation between increased DNA copy number and gene expression level.

Bermont teaches that overexpression of p185 is usually associated with c-erbB-2 amplification. Specifically, Bermont reports that 100% of the overexpressed p185 protein in 106 breast cancer samples studied also displayed c-erbB-2 amplification. *See* Bermont *et al.,* "Relevance of p185 HER-2/neu oncoprotein quantification in human primary breast carcinoma." *Breast Cancer Res Treat.* 2000 63(2):163-9 (submitted herewith). *See also* Hu *et al.*, "Profiling of differentially expressed cancer-related genes in esophageal squamous cell carcinoma (ESCC) using human cancer cDNA arrays: overexpression of oncogene MET correlates with tumor differentiation in ESCC." *Clin Cancer Res.* 2001 7(11):3519-25 (the results of cDNA arrays showed that 13 cancer-related genes were upregulated > or = 2 fold and immunostaining results of the expression of the MET gene showed MET overexpression at the protein level, validating the cDNA arrays findings) (submitted herewith).

Papotti *et al.* (Diagn Mol Pathol. 9(1):47-57 (2000); submitted herewith) studied the somatostatin type 2 receptor (sst2) in 26 different neuroendocrine lung tumors. They investigated mRNA levels by RT-PCR and protein levels by immunohistochemistry

using 2 different antibodies. The authors report that "in the majority of samples a good correlation between sst2 mRNA (as detected by RT-PCR) and sst2 protein expression (as detected by immunohistochemistry) was observed" (Abstract). The authors also performed *in situ* hybridization (ISH) in selected samples which "paralleled the results obtained with the other techniques" (Abstract).

Walmer *et al.* (Cancer Res. 55(5):1168-75 (1995); submitted herewith) looked at lactoferrin mRNA and protein expression in endometrial adenocarcinomas and report that two thirds (8 of 12) of the samples examined overexpress lactoferrin. Walmer *et al.* also found that "this tumor-associated increase in lactoferrin expression includes an elevation in the mRNA and protein of individual cells" and that "serial sections of malignant specimens show(ed) a good correlation between the localization of lactoferrin mRNA and protein in individual epithelial cells by *in situ* RNA hybridization and immunohistochemistry" (Abstract).

Janssens *et al.* (Tumour Biol. 25(4):161-71 (2004); Submitted herewith) evaluated the involvement of frizzled receptors (Fzds) in oncogenesis. They investigated mRNA expression levels in 30 different human tumor samples and their corresponding (matched) normal tissue samples by real-time quantitative PCR. Janssens *et al.* observed markedly increased Fzd5 mRNA levels in 8 of 11 renal carcinoma samples and that "Western blot analysis of crude membrane fractions revealed that Fzd5 protein expression in the matched tumor/ normal kidney samples correlated with the observed mRNA level" (Abstract).

Hahnel *et al.* (Breast Cancer Res Treat. 24(1):71-4 (1992); submitted herewith) studied expression of the pS2 gene in breast tissues by measuring mRNA levels using Northern blotting and protein levels by radioimmunoassay. Hahnel *et al.* indicate that "there was a good correlation between the two measurements, indicating that expression of the pS2 gene in breast tissues may be assessed by either method."

Kammori *et al.* (Int J Oncol.. 27:1257-63 (2005); submitted herewith) studied the expression of human telomerase reverse transcriptase (hTERT) gene and protein (besides estrogen and progesterone receptors) in breast tumors using *in situ*

hybridization (ISH) for mRNA and immunohistochemistry (IHC) for the protein. They looked at 64 adenocarcinomas, 2 phyllode tumors and their adjacent normal breast tissues and found that hTERT mRNA was detected in 56 tumors but in neither of the 2 phyllode tumors whereas hTERT protein expression was detected by IHC in 52 tumors but in neither of the 2 phyllode tumors. The authors concluded that "there was a strong correlation between detection of hTERT gene expression by ISH and of hTERT protein by ICH in tissue specimens from breast tumors" (Abstract).

Maruyama *et al.* (Am. J. Pathol. 155:815-822 (1999); submitted herewith) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that all three Id mRNA species were expressed at high levels in pancreatic cancer cells as compared to normal or CP samples, and that the pancreatic cancer cell lines also exhibited "a good correlation between Id mRNA and protein levels" (Abstract). The authors measured both mRNA and protein expression in five different human pancreatic cancer cell lines. The authors observed a correlation between mRNA and protein expression of Id1 in all five cell lines, and a correlation between mRNA and protein expression for Id2 and Id3 in four out of five cell lines. In these discordant cases, Id protein levels were increased while mRNA levels were not. As noted above, Applicants make no assertions regarding changes in protein levels when mRNA levels are unchanged, nor does evidence of changes in protein levels when mRNA levels are unchanged have any relevance to Applicants' asserted utility. Thus, the authors report that increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Bea *et al.* (Cancer Res. 61:2409-2412 (2001); submitted herewith) investigated gene amplification, mRNA expression, and protein expression of the putative oncogene BMI-1 in lymphoma samples. The authors examined BMI-1 protein expression in 31 tumors for which levels of gene amplification and mRNA expression had been determined. Bea *et al.* found that "[a] good correlation between BMI-1 mRNA levels and protein expression was observed in all types of lymphomas" (Abstract). Thus, the authors report that increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Applicants also submit an additional reference in support of the assertion that in general, mRNA expression levels are correlated with protein expression levels. Futcher *et al.* (Mol. Cell. Biol. 19:7357-7368 (1999) (submitted herewith)) analyzed the yeast proteome using 2D gel electrophoresis, gathering quantitative data on protein abundance for about 1,400 spots. This data was compared to mRNA abundance for each gene as determined both by SAGE (serial analysis of gene expression) and by hybridization of cRNA to oligonucleotide arrays. The authors concluded that "several statistical methods show a strong and significant correlation between mRNA abundance and protein abundance" (page 7360, col. 2; emphasis added).

The authors note that Gygi *et al.*, cited in the Office action and discussed more fully below, completed a similar study that generated broadly similar data, but reached different conclusions. Futcher *et al.* note that this is in part a difference in viewpoint, in that "Gygi *et al.* focus on the fact that the correlations of mRNA and codon bias with protein abundance are far from perfect" (page 7367, col. 1). Applicants respectfully submit that a showing that mRNA levels can be used to "accurately predict" the precise levels of protein expression is not required. Applicants need only show that there is a correlation between mRNA and protein levels, such that mRNA overexpression generally predicts protein overexpression. The data of both Futcher *et al.* and Gygi *et al.* clearly meets this standard.

Futcher *et al.* also point out that "the different conclusions are also partly due to different methods of statistical analysis, and to real differences in data." Futcher *et al.* first note that Gygi *et al.* used the Pearson product-moment correlation coefficient (r_p) to measure the covariance of mRNA and protein abundance. Futcher *et al.* point out that "the r_p correlation is a parametric statistic and so requires variates following a bivariate normal distribution; that is, it would be valid only if both mRNA and protein abundances were normally distributed" (page 7367, col. 1; emphasis added). As the authors disclose, "both distributions are very far from normal," and thus "a calculation of r_p is inappropriate" (page 7367, col. 1).

In contrast, Futcher *et al.* used two different statistical approaches to determining the correlation between mRNA and protein abundances. First, they used the Spearman rank correlation coefficient (r_s), an nonparametric statistic that does not require the data to be normally distributed. Using the r_s, the authors found that mRNA abundance was well correlated with protein abundance ($r_s = 0.74$). Applying this statistical approach to the data of Gygi *et al.* also resulted in a good correlation ($r_s = 0.59$), although the correlation was not quite as strong as for the Futcher *et al.* data. In a second approach, Futcher *et al.* transformed the mRNA and protein data to forms where they were normally distributed, in order to allow calculation of an r_p. Two types of transformation (Box-Cox and logarithmic) were used, and both resulted in good correlations between mRNA and protein abundance for Futcher *et al.*'s data.

Futcher *et al.* also note that the two studies used different methods of measuring protein abundance. Gygi *et al.* cut spots out of each gel and measured the radiation in each spot by scintillation counting, whereas Futcher *et al.* used phosphorimaging of intact gels coupled to image analysis. Futcher *et al.* point out that Gygi *et al.* may have systematically overestimated the amount of the lowest-abundance proteins, because of the difficulty in accurately cutting out very small spots from the gel, and because of difficulties in background subtraction for small, weak spots. In addition, Futcher *et al.* note that they used both SAGE data and RNA hybridization data to determine mRNA abundances, which is most helpful to accurately measure the least abundant mRNAs. As a result, while the Futcher data set "maintains a good correlation between mRNA and protein abundance even at low protein abundance" (page 7367, col. 2), the Gygi data shows a strong correlation for the most abundant proteins, but a poor correlation for the least abundant proteins in their data set. Futcher *et al.* conclude that "the poor correlation of protein to mRNA for the nonabundant proteins of Gygi *et al.* may reflect difficulty in accurately measuring these nonabundant proteins and mRNAs, rather than indicating a truly poor correlation *in vivo*" (page 7367, col. 2).

Accordingly, the results of Futcher *et al.* demonstrate "a strong and significant correlation between mRNA abundance and protein abundance" (page 7360, col. 2). Further, Futcher *et al.* show that when corrected for an inappropriate statistical analysis

and systematic error in the measurement of low abundance proteins, the data of Gygi *et al.* also meets the "more likely than not standard" and shows that a positive correlation exists between mRNA levels and protein levels.

Thus, although there may not always be a 100% correlation between gene amplification and protein overexpression, the above-discussed references evidence that the utility of the present invention does not violate any scientific principle, nor is it wholly inconsistent with the knowledge in the art. Therefore, the above-discussed references evidence that one of ordinary skill in the art would find it more likely than not that gene amplification of PRO357 correlates with overexpression of the PRO357 polypeptide and would accept the asserted diagnostic utility of the claimed antibodies as a specific, substantial, and credible utility.

The Totality of the Evidence Demonstrates that it is More Likely than Not that One of Ordinary Skill in the Art Would Accept that Generally Gene Amplification Correlates with Protein Overexpression

Applicants respectfully submit that the Office action fails to set forth a prima facie case of lack of utility. However, even if the Office maintains that a prima facie case of lack of utility is established, as discussed below, consideration of the totality of the evidence, including the evidence presented by Applicants as discussed above, and the references cited by the Office, which are discussed below, clearly demonstrates that the one of ordinary skill in the art would not find it more likely than not that, in general, there is no correlation between gene amplification and protein overexpression. Indeed, the totality of the evidence shows that the proposition that there will be correlation between protein and transcript levels does not violate any scientific principles nor is it wholly inconsistent with knowledge in the art.

In support of the position that correlations between gene amplification and protein overexpression cannot be presumed, the Office relies on several references including, Pennica, Konopka, Chen, Hu, LaBaer, Haynes, Gygi, Lian, Fessler, and Hanish. However, none of these references, either alone or in combination, demonstrate that the assertion that gene amplification correlates with protein overexpression violates a

scientific principle or is *wholly* inconsistent with contemporary knowledge in the art. Indeed, the cited references do not make it more likely than not that one of ordinary skill in the art would find that, in general there is no correlation between gene amplification and protein overexpression.

Specifically, Pennica *et al.* is relied on by the Office for the teaching that:

> An analysis of *WISP-1,* gene amplification and expression in human colon tumors showed a correlation between DNA amplification and overexpression, whereas overexpression of *WISP-3* RNA was seen in the absence of DNA amplification. In contrast, *WISP-2* DNA was amplified in the colon tumors, but its mRNA expression was significantly reduced in the majority of tumors compared with the expression in normal colonic mucosa from the same patient.

Although the above cited passage from Pennica may illustrate that increased copy number does not *necessarily* result in increased polypeptide expression., Pennica *et al.* does not teach that no correlation can be presumed. Moreover, the standard for determining whether a correlation can be presumed is not absolute certainty. Rather, Applicants only must show that the existence of a correlation between gene amplification and protein overexpression is generally more likely than not. The fact that in Pennica, a case focused on a specific class of closely related molecules, there seemed to be no correlation with gene amplification and the level of mRNA/protein expression does not establish that it is more likely than not, in general, that such correlation does not exist. The Office action fails to show whether the lack or correlation observed for the family of WISP polypeptides is typical, or is merely a discrepancy, an exception to the rule of correlation. Indeed, the working hypothesis among those skilled in the art is that, if a gene is amplified in cancer, the encoded protein is likely to be expressed at an elevated level. In fact, as noted even in Pennica *et al.*, "[a]n analysis of *WISP*-1 gene amplification and expression in human colon tumors *showed a correlation between DNA amplification and over-expression* . . . ." (Pennica *et al.*, page14722, left column, first full paragraph, emphasis added).

More specifically, Pennica et al. noted that *WISPs-1* and *2* had copy numbers that were significantly higher than one, indicating gene amplification. Pennica *et al.* further noted

that the copy number for *WISP-3* was "indistinguishable" from one (p=1.666), indicating no or minimal gene amplification. Next, Pennica *et al.* examined the levels of *WISP* transcripts in RNA isolated from 19 adenocarcinomas and their matched normal mucosa using quantitative PCR. Pennica *et al.* found that *WISP-1* RNA levels displayed *good correlation* to gene amplification of *WISP-1.* Specifically, Pennica *et al.* found that RNA levels of *WISP-1* in tumor tissue were significantly increased in 84% (16/19) of the human colon tumors examined when compared with normal adjacent mucosa. *See* page 14721, Figure 7.

However, Pennica *et al.* also found that *WISP-3* RNA levels did not significantly correlate with *WISP-3* gene amplification. In particular, although *WISP-3* did not display significant gene amplification levels, RNA levels in tumor tissue were overexpressed in 63% (12/19) of the human colon tumors examined when compared with normal adjacent mucosa.

Further, Pennica *et al.* also report that *WISP-2* gene amplification levels are inversely correlated with RNA expression levels. That is, although *WISP-2* was significantly amplified, RNA levels of *WISP-2* in tumor tissues were significantly lower than RNA levels of *WISP-2* in normal adjacent mucosa. Specifically, 79% (15/19) of the samples examined displayed this expression pattern.

The Office action relies on this last result as support for the proposition that one of ordinary skill in the art would not expect gene amplification levels to correlate with protein overexpression absent explicit evidence of protein overexpression. Applicants respectfully disagree for three reasons. First, *WISP-1* gene amplification and RNA expression levels showed a significant positive correlation. Second, although *WISP-3* was not significantly amplified, it was amplified (P=1.666) and significantly overexpressed. Third, although *WISP-2* gene amplification and RNA expression levels seemed to be inversely related, Pennica *et al.* state that this result might be inaccurate. Specifically, Pennica *et al.* suggest that "[b]ecause the center of the 20q13 amplicon has not yet been identified, it is possible that the apparent amplification observed for *WISP-2* may be caused by another gene in this amplicon." *See* 14722. Thus, because

the RNA expression pattern of *WISP-2* cannot be accurately attributed to gene amplification of *WISP-2,* this result should be disregarded. Therefore, particularly in light of the references discussed above, one of ordinary skill in the art may conclude that Pennica *et al.* supports a utility for the present invention because Pennica *et al.* teaches that gene amplification of *WISP-1* strongly correlates (84%) with RNA overexpression.

Arguably then Pennica *et al.* teaches nothing conclusive regarding the absence of correlation between amplification of a gene and over-expression of the encoded WISP polypeptide. More importantly, the teaching of Pennica *et al.* is specific to *WISP* genes. Pennica *et al.* has no teaching whatsoever about the correlation of gene amplification and protein expression in general.

The Office action also relies on Konopka *et al.,* and alleges this reference teaches that gene amplification does not correlate with protein overexpression. Applicants respectfully disagree with the Office's characterization of the teaching of Konopka and submit that the Office has generalized a very specific result disclosed by Konopka *et al.* to cover all genes. Konopka *et al.* actually state that "[p]rotein expression is not related to amplification of the *abl* gene but to variation in the level of *bcr-abl* mRNA produced from a single Ph^1 template." (See Konopka *et al.*, Abstract, emphasis added). The paper does not teach anything whatsoever about the correlation of protein expression and gene amplification in general, and provides no basis for the generalization that apparently underlies the present rejection. The statement of Konopka *et al.* that "[p]rotein expression is not related to amplification of the *abl* gene . . ." is not sufficient to establish a *prima facie* case of lack of utility. It is not enough to show that for a particular gene a correlation does not exist. The law requires that the Examiner show evidence that it is more likely than not that such correlation, in general, does not exist. Such a showing has not been made.

Further, the combined teachings of Pennica *et al.* and Konopka *et al.* are not directed towards genes in general but to a single gene or genes within a single family and thus,

their teachings cannot support a general conclusion regarding correlation between gene amplification and mRNA or protein levels.

In further support of its position, the Office action relies on Chen *et al.* and argues that Chen clearly teaches that "the use of mRNA expression patterns by themselves, however, is insufficient for understanding the expression of protein products . . . [and] it is not possible to predict overall protein expression levels based on average mRNA abundance in lung cancer samples." Page 8 of the Office action mailed 2/27/06. Although the Chen reference examines correlation between gene amplification and protein overexpression in human lung adenocarcinomas, the teachings of Chen do not make it more likely than not that one of ordinary skill in the art would doubt the truth of Applicants' assertion of utility. Specifically, Chen "*suggests* that it is not possible to predict *overall* protein expression levels based on *average* mRNA abundance in lung cancer samples." Chen *et al.,* "Discordant Protein and mRNA Expression in Lung Adenocarcinomas," *Mol.& Cellular Proteomics 1.4.* 2002. 304-313, 311-12. However, Applicants assertion of utility is not based on *overall* protein expression levels or *average* mRNA abundance in lung cancer samples. Moreover, even if Chen does suggest that in general gene amplification levels cannot be used to predict protein expression levels, this teaching of Chen alone is not sufficient to overcome the teachings of the specification and the evidence submitted by Applicants.

Indeed, Applicants assertion of utility is based on the general principle that gene amplification correlates with protein overexpression. This principle is supported by numerous articles discussed above and three expert declarations submitted herewith. For example, Applicants assertion is based on the gene amplification demonstrated and measured in Example 28 of the specification. This gene amplification has been demonstrated to correlate with protein overexpression in 28 of 31 samples tested. *See e.g.* the Polakis Declarations I and II, submitted herewith and discussed above (providing declaratory evidence that when gene amplification as described in Example 28 of the specification was observed in the Tumor Antigen Project, a project which led to the development of the PRO357 molecules, 80% of the time the gene amplification was found to correlate with a change in gene-product (*i.e.* protein) expression levels).

The Office action also cites Hu *et al.* because "Hu et al discovered that, for genes displaying a 5-fold change or less in tumors compared to normal, there was no evidence of a correlation between altered gene expression and a known role in the disease. However, among genes with a 10-fold or more change in expression level, there was a strong and significant correlation." Page 8 of the Office action mailed 2/27/06. The Office action also cites another article by an author of the Hu reference, Dr. LaBaer, for the same proposition.

However, the La Baer reference and the Hu *et al.* reference entitled "Analysis of Genomic and Proteomic Data using Advanced Literature Mining" (emphasis added), drew conclusions based upon statistical analysis of information obtained from published literature, and not from experimental data. Nowhere does either article discuss any information on microarray experiments, for example, the control used in the assays. In addition, both references have only assessed the biological significance of genes identified by microarray assay solely based on the frequency of literature citations of these genes, which does not reflect the true biological significance of these genes. Therefore, the statistical analysis by Hu *et al.,* and LaBaer is not reliable and informative.

The Office action further cites several references in support of the proposition that transcript levels do not correlate with polypeptide levels in normal tissues. Specifically, in support of this position, the Office action relies on references by Haynes *et al,* Gygi *et al.,* Lian *et al.,* Fessler *et al.,* and Hanish. Applicants respectfully submit that none of these references make it more likely than not that one of ordinary skill in the art would conclude that in general no correlation exists between gene amplification and protein overexpression levels.

More specifically, the Office action relies on Haynes and Gygi for the proposition that abundance of mRNA expression levels does not necessarily result in increased protein expression levels. Haynes and Gygi are related references based on results obtained from "the mRNA and protein levels of a group of genes expressed in exponentially growing cells of the yeast *S. cerevisiae." See* Haynes, *et al.,* "Proteome analysis:

Biological assay or data archive?" *Electrophoresis,* 1998. 19:1862-1871; Gygi *et al.,* "Correlation between Protein and mRNA Abundance in Yeast," *Molecular and Cellular Biology.* 1999. 19(3): 1720-1730. Specifically, Haynes and Gygi "explore a quantitative comparison of mRNA transcript and protein levels for a relatively large number of (yeast) genes expressed in the same metabolic state." *See* Gygi *et al.,* at 1720; Haynes *et al.,* at 1862.

As an initial matter, the results of Haynes and Gygi are not relevant here because they were not obtained in a human system, did not examine any particular human gene or protein expression, and most significantly, did not examine any genes that are amplified in a cancerous state. Instead, Haynes and Gygi both examine the ability to predict protein expression levels in a *biological system.* Specifically, Haynes and Gygi examine whether there is an overall *system* correlation between gene and protein expression levels.

In contrast, the present invention involves the correlation between expression levels of a single gene, the PRO357 nucleic acid, and its encoded polypeptide. PRO357 nucleic acid is amplified in a diseased system, lung and colon tumors.

In any event, even if Haynes and Gygi were a comparable system, both report that "[f]or the entire group (106 genes) for which a complete data set was generated, there was a *general trend of increased protein levels resulting from increased mRNA levels."* Gygi *et al.,* at 1726 (emphasis added); Haynes *et al.,* 1863. In fact, Gygi reports that the Pearson product moment correlation coefficient for the whole data set was 0.935. Gygi *et al.,* at 1726. The Office action however, ignores this overall correlation pattern, which supports the utility of the present invention, and seizes upon a subset of genes studied in Gygi.

However, even if the general correlation taught by Haynes and Gygi is rejected, Applicants disagree that the data based on the "subset of genes" the Office action focuses on teach one of ordinary skill in the art that the present invention is not supported by a utility. Specifically, Gygi *et al.,* separate the genes studied into two groups: (1) those with a message level below 10 copies / cell in a healthy system and

(2) those with more than 10 copies for cell. The Office action focuses on the first group of genes, but the second group is more relevant to the present invention, which is directed to a polypeptide encoded by an amplified nucleic acid. Indeed, this second group of genes Gygi *et al.,* studied demonstrated a high correlation between the high message levels of those genes and high protein expression levels. *See* Gygi, *et al.,* at 1726, 1727 (Figure 6). In addition, Gygi *et al.,* note that due to lower message levels in the first group (*i.e.* genes with message levels of below 10 copies / cell) "the error associated with these values (correlation between message and protein levels) may be quite large." Gygi at 1728.

Moreover, as discussed above, the Futcher reference discusses an analysis that is nearly identical to the analysis carried out by Gygi but reaches a different conclusion. Specifically, Futcher concludes that gene amplification levels generally are predictive of protein overexpression. As explained above, Futcher attributes this difference in conclusions to a difference in view point, use of different methods of statistical analysis, and real differences in data.

Thus, neither Haynes nor Gygi support the rejection of claims 22-26. Rather, Haynes and Gygi teach that <u>in general</u> there is a good correlation between message and protein levels for genes with high copy numbers per cell, which supports the asserted utility of the present invention.

In further support of the alleged lack of correlation between mRNA expression and protein expression levels, the Office action cites Lian *et al.* for the statement that there is a poor correlation between mRNA expression and protein abundance in mouse cells, and therefore it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels. Page 9 of the Office action mailed 2/27/06.

In Lian *et al.*, the authors looked at the mRNA and protein levels of genes in a derived promyelocytic mouse cell-line during differentiation of the cells from a promyelocytic stage of development to mature neutrophils following treatment with retinoic acid. The level of mRNA expression was measured using 3'-end differential display (DD) and oligonucleotide chip array hybridization to examine the expression of genes at 0, 24, 48

and 72 hours after treatment with retinoic acid. Protein levels were qualitatively assessed at 0 and 72 hours after retinoic acid treatment following 2-dimensional gel electrophoresis.

Lian *et al.* report that they were able to identify 28 proteins which they considered differentially expressed (page 521). Of those 28, only 18 had corresponding gene expression information, and only 13 had measurable levels of mRNA expression (page 521, Table 6). The authors then compared the qualitative protein level from the 2-D electrophoresis gel to the corresponding mRNA level, and reported that only 4 genes of the 18 present in the database had expression levels which were consistent with protein levels (page 521, col. 1). The authors note that "[n]one of these was on the list of genes that were differentially expressed significantly (5-fold or greater change by array or 2-fold or greater change by DD)" (page 521; emphasis added). Based on these data, the authors conclude "[f]or protein levels based on estimated intensity of Coomassie dye staining in 2DE, there was poor correlation between changes in mRNA levels and estimated protein levels" (page 522, col. 2).

The authors themselves admit that there are a number of problems with the data presented in this reference. At page 520 of this article, the authors explicitly express their concerns by stating that "[t]hese data must be considered with several caveats: membrane and other hydrophobic proteins and very basic proteins are not well displayed by the standard 2DE approach, and proteins presented at low level will be missed. In addition, to simplify MS analysis, we used a Coomassie dye stain rather than silver to visualize proteins, and this decreased the sensitivity of detection of minor proteins." (emphasis added). It is known in the art that Coomassie dye stain is a very insensitive method of measuring protein. This suggests that the authors relied on a very insensitive measurement of the proteins studied. The conclusions based on such measurements can hardly be accurate or generally applicable. In particular, the total number of proteins examined by Lian *et al.* was only 50 (page 520, col. 2), as compared to the approximately 7000 genes for which mRNA levels were measured (page 515, col. 1). Thus, the conclusions are based on a very small and atypical set of proteins.

Applicants also emphasize that Applicants are asserting that a measurable change in mRNA level generally leads to a corresponding change in the level of protein expression, not that changes in protein level can be used to predict changes in mRNA level. As discussed above, Lian *et al.* did not take genes which showed significant mRNA changes and check the corresponding protein levels. Instead, the authors looked at a small and unrepresentative number of proteins, and checked the corresponding mRNA levels. Based on the authors' criteria, mRNA levels were significantly changed if they were at least 5-fold different when measured using a microchip array, or 2-fold different when using the more sensitive 3'-end differential display (DD). Of the 28 proteins listed in Table 6, only one has an mRNA level measured by microarray which is differentially expressed according to the authors (spot 7: melanoma X-actin, for which mRNA changed from 2539 to 341.3, and protein changed from 1 to 3). None of the other mRNAs listed in Table 6 show a significant change in expression level when using the criteria established by the authors for the less sensitive microarray technique.

There is also one gene in Table 6 whose expression was measured by the more sensitive technique of DD, and its level increased from a qualitative value of 0 to 2, a more than 2-fold increase (spot 2: actin, gamma, cytoplasmic). This increase in mRNA was accompanied by a corresponding increase in protein level, from 3 to 6.

Therefore, although the authors characterize the mRNA and protein levels as having a "poor correlation," this does not reflect a lack of a correlation between a change in mRNA level and a corresponding change in protein level. Only two genes meet the authors' criteria for differentially expressed mRNA level, and of those, one apparently shows a corresponding change in protein level and one does not. Thus, there is little basis for the authors' conclusion that "it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels (as estimated from 2DE)."

In further support of the alleged lack of correlation between mRNA expression and protein expression levels, the Office action also cites a publication by Fessler *et al.*, as

having "found a 'poor concordance between mRNA transcript and protein expression changes' in human cells." Pages 8-9 of the Office action mailed 2/27/06. Fessler is not contrary to Applicants' asserted utility, and actually supports Applicants' assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein. As noted above, Applicants make no assertions regarding changes in protein levels when mRNA levels are unchanged, nor does evidence of changes in protein levels when mRNA levels are unchanged have any relevance to Applicants' asserted utility.

Fessler *et al.* studied changes in neutrophil (PMN) gene transcription and protein expression following lipoplysaccharide (LPS) exposure. In Table VIII, Fessler *et al.* list a comparison of the change in the level of mRNA for 13 up-regulated proteins and 5 down-regulated proteins. Of the 13 up-regulated proteins, a change in mRNA levels is reported for only 3 such proteins. For these 3, mRNA levels are increased in 2 and decreased in the third. Of the 5 down-regulated proteins, a change in mRNA is reported for 3 such proteins. In all 3, mRNA levels also are decreased. Thus, in 5 of the 6 cases for which a change in mRNA levels are reported, the change in the level of mRNA corresponds to the change in the level of the protein. This is consistent with Applicants' assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein.

Regarding the remainder of the proteins listed in Table VIII, in 6 instances, protein levels changed while mRNA levels were unchanged. This evidence has no relevance to Applicants' assertion that changes in mRNA levels lead to corresponding changes in protein levels, since Applicants are not asserting that changes in mRNA levels are the only cause of changes in protein levels. In the final 6 instances listed in Table VIII, protein levels changed while mRNA was noted as "absent." This evidence also has no relevance to Applicants' assertion that changes in mRNA levels causes corresponding changes in protein levels. By virtue of being "absent," it is not possible to tell whether mRNA levels were increased, decreased or remained unchanged in PMN upon contact with LPS. Nothing in these results by Fessler *et al.* suggests that a change in the level of mRNA for a particular protein does not generally lead to a corresponding change in

the level of the encoded protein. Accordingly, these results are not contrary to Applicants' assertions.

The Office action points to Fessler's statement regarding Table VIII that there was "a poor concordance between mRNA transcript and protein expression changes." Page 10 of the Office action mailed 2/27/06. As is clear from the above discussion, this statement does not relate to a lack of correlation between a change in mRNA levels leading to a change in protein levels, because in 5 of 6 such instances, changes in mRNA and protein levels correlated well. Instead, this statement relates to observations in which protein levels changed when mRNA was either unchanged or "absent." As such, this statement is an observation that in addition to transcriptional activity, LPS also has post-transcriptional and possibly post-translational activity that affect protein levels, an observation which is not contrary to Applicants' assertions. Accordingly, Fessler's results are consistent with Applicants' assertion that a change in mRNA level of for a particular protein generally leads to a corresponding change in the level of the encoded protein, since 5 of 6 genes demonstrated such a correlation.

Finally, as further support, the Office action cites Hanash as teaching that "a gene can be amplified 100-fold in certain tumors with no demonstrable effect on RNA levels for that gene." Page 10 of the Office action mailed 2/27/06. Hanash is also cited for the position that "protein levels can be increased, decreased, or modified with no demonstrable changes in the levels of their corresponding RNAs." However, as with the Lian and Fessler references, Hanash examines protein levels and attempts to discern whether those protein levels correlate with mRNA and gene levels. As stated above, Applicants assertion of utility is based on Applicants' assertion that a change in mRNA level of for a particular protein generally leads to a corresponding change in the level of the encoded protein.

Further, the statement from Hanash that "no single type of molecular approach fully elucidates tumor behavior, necessitating analysis at multiple levels encompassing genomics and proteomics," does not contradict the evidence discussed above and submitted herewith. As an initial matter, statistical certainty is not required, rather the

asserted utility must not violate or be wholly inconsistent with any scientific principle. As shown in the above discussion, Applicants assertion of utility based on a correlation between gene amplification and protein overexpression levels in lung and colon cancer tissues does not violate, nor is it inconsistent with any scientific principle. In addition, the Second Declaration of Paul Polakis submitted herewith, provides direct evidentiary proof that of 31 genes identified as being detectably overexpressed in human tumor tissue, 28 of those genes, *i.e.,* greater than 90%, were also detectably overexpressed in human tumor tissue at the protein level.

For the reasons given above, Applicants respectfully submit that the Office action does not establish a *prima facie* showing of lack of utility. Therefore, the Patent Office has failed to meet its initial burden of proof. Furthermore, even if the Office maintains that a *prima facie* case of lack of utility is established, consideration of the totality of the evidence clearly demonstrates that Applicants asserted utility is specific, substantial, and credible. Moreover, even if the Office maintains that under consideration of the totality of the evidence there is no correlation between gene amplification and increased mRNA/protein expression, (which Applicants expressly do not concede), a polypeptide encoded by a gene that is amplified in cancer would still have a specific, substantial, and credible utility as evidenced by the Declaration of Avi Ashkenazi, Ph.D., submitted herewith as Exhibit E. Specifically, at paragraph 6, Dr. Ashkenazi declares:

> [E]ven when amplification of a cancer marker gene does not result in significant over-expression of the corresponding gene product, this very absence of gene product over-expression of the corresponding gene product still provides significant information for cancer diagnosis and treatment. Thus, if over-expression of the gene product does not parallel gene amplification in certain tumor types but does so in others, then parallel monitoring of gene amplification and gene product over-expression enables more accurate tumor classification and hence better determination of suitable therapy. In addition, absence of over-expression is crucial information for the practicing clinician. If a gene is amplified but the corresponding gene product is not over-expressed, the clinician accordingly will decide not to treat a patient with agents that target that gene product.

The Ashkenazi Declaration is further supported by the teachings of Hanna and Mornin. *See* (Pathology Associates Medical Laboratories, August (1999), submitted herewith).

Specifically, both the Ashkenazi Declaration and the Hanna and Mornin article illustrate that simultaneous testing of gene amplification and gene product over-expression enables more accurate tumor classification, even if the gene-product, the protein, is not over-expressed. This leads to better determination of a suitable therapy for the tumor as demonstrated by the real-world example of the breast cancer marker HER-2/neu. Hence, Applicants respectfully submit that this utility is sufficient to satisfy the utility requirement even if the Office maintains that gene amplification does not correlate with protein overexpression. Significantly, the law only requires that an applicant provide "one credible assertion of a specific and substantial utility for each claimed invention to satisfy the utility requirement." *See* MPEP § 2107 (emphasis added). Applicants have overcome this ground of rejection for the reasons discussed above and respectfully request that this ground of rejection be withdrawn.

Rejection under 35 U.S.C. § 112, first paragraph:

Enablement

The Examiner contends that because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility, one skilled in the art would not know how to use the claimed invention.

Applicants respectfully disagree. As discussed above, the claimed invention is adequately supported by an asserted utility that is both specific and substantial. Applicants respectfully request the Examiner reconsider and withdraw the rejection of the claims under 35 U.S.C. § 112 ¶1 for alleged inadequate disclosure on how to use the claimed invention.

Rejection under 35 U.S.C. § 112, second paragraph:

The Office action alleges that claims 22-26 are indefinite. The Examiner kindly points out that this rejection could be overcome by amending the last two lines of claim 22 to recite "and diagnosing said subject with lung or colon cancer if the presence of said polypeptide is detected." Applicants thank the Examiner and have herein amended

claim 22 per the Examiner's suggestion. No new matter is added by this amendment and Applicants submit this ground of rejection is overcome. Applicants therefore respectfully request that it be withdrawn.

CONCLUSION

Applicants believe this Amendment and Request for Reconsideration fully responds to the Office Action. Applicants respectfully request the Examiner grant early allowance of this application. The Examiner is invited to contact the undersigned attorney for the Applicant via telephone if such communication would expedite this application.

Applicants believe no fee is due in connection with the filing of this Request for Reconsideration, however, should any fees be deemed necessary for any reason relating to this paper, the Commissioner is hereby authorized to deduct said fees from Brinks Hofer Gilson & Lione Deposit Account No. 23-1925. A duplicate copy of this document is enclosed.

Respectfully submitted,

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